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A Comprehensive Review of Different Liver Toxicants Used in Experimental Pharmacology

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ABSTRACT
Liver is a primary organ involved in metabolism of food and drugs. Liver disorders are mainly caused by toxic chemicals, such as antibiotics, chemotherapeutic agents, peroxidised oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbons etc. Toxic liver injury produced by drugs and chemicals may virtually mimic any form of naturally occurring liver disease. In view of multiplicity and complexity of the liver functions, it is obvious that no single test can establish the disturbances in liver function. Thus, a battery of liver function tests is employed for accurate diagnosis, to assess the severity of damage, to judge prognosis and to evaluate therapy. To study hepatoprotective potential of any herbal product, isolated phytochemical or synthetically developed moiety induction of experimental liver damage is prerequisite. The selection of toxicant depends on type and nature of liver damage required as every toxicant has its unique mode of action producing specific type of destruction. The hepatoprotective mechanism of any drug under study can be explained based on its protective behavior against different toxicants. This review is precise compilation of experimental liver pharmacology taking concern dose, route, and schedule of different liver toxicants along with evaluation biochemical, histological and functional ability parameters. The literature compilation will definitely help researcher in design and assessment of studies involving liver function.

Keywords: Hepatotoxicity, liver toxicants, biochemical parameters, liver function tests, liver histopathology, bromosulphthalein.

INTRODUCTION

The liver is the largest organ in the body and serves many vital functions such as remove damaged red blood cells from the blood in co-ordination with spleen, produces bile, clotting factors, stores vitamins, minerals, protein, fats and glucose from diet. [1-2] The most important task of the liver is to filter alcohol,
chemotherapeutic drugs, antibiotics and toxicants. If accumulation of toxins is faster than the liver metabolizing ability, hepatic damage may occur.
Besides the chemicals and toxicant, there are several factors that increase the risk of hepatic injury that includes different races, as blacks and Hispanics may be more susceptible to isoniazid toxicity, alcohol ingestion, elderly persons have increased risk of hepatic injury because of decreased clearance, drug to drug interactions and reduced hepatic blood flow. Females are more prone to hepatic injury.

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Unique gene encodes in each P_{450} protein, long acting drugs, host factors, persons suffering from AIDS, malnourished and fasting person may be susceptible to hepatic injury because of low glutathione stores. [3]
The liver metabolizes virtually every drug or toxin introduced in the body. Metabolism of drugs or toxins occurs in 2 phases. [4] In the phase 1 reaction, the drug is made polar by oxidation or hydroxylation. All drugs may not undergo this step, and some may directly undergo the phase 2 reaction. The cytochrome P_{450} enzymes catalyze phase 1 reactions. [5-6] Most of these intermediate products are transient and highly reactive. These reactions may result in the formation of metabolites that are far more toxic than the parent substrate and may result in liver injury. Phase 2 reactions may involve conjugation with a moiety (i.e., acetate, amino acid, sulfate, glutathione, glucuronic acid). [7] Subsequently, drugs with high molecular weight may be excreted in bile, while the kidneys excrete the smaller water like
primidone,
ethanol, glucocorticoids, rifampin, griseofulvin, quinine and omeprazole induces P_{450} enzyme activity, whereas in

contrast, amiodarone, cimetidine, erythromycin, isoniazid, ketoconazole, metronidazole, sulfonamides and quinidine inhibit the P_{450} enzyme.

Some hepatotoxins found in nature are the products of plants (e.g. albitocin, cycasin, pyrrolizidines, safrole, tannic acid, indospicine), fungal (e.g. aflatoxin, phalloidin, luteoskyrin) [8] or bacterial metabolism (e.g. *Corynebacterium diptheriae*, *Clostridium botulinus, Streptococcus hemolyticus* and some stains of *E. coli*) or minerals. Many hepatotoxic agents are products of chemicals or pharmaceutical industry. Others are industrial byproducts or waste materials that by polluting the environment may cause hepatotoxicity in humans as well as animals. [9] Hepatic injury can have several forms, classified as:

a. Acute hepatitis or chronic hepatitis (inflammatory liver disease)
b. Hepatosis (non inflammatory disease)
c. Cirrhosis (degenerative disorder resulting in fibrosis of the liver)

Some agents interfere with bile secretion with little or no overt injury to hepatic parenchyma. Other leads to necrosis, cirrhosis or carcinoma. Some toxicants produce curious degeneration or vascular lesion. Drugs or chemicals can produce the entire range of known hepatic lesion.

MECHANISMS OF HEPATIC INJURY

The mechanism of hepatic injury can be categorized as pathophysilogical mechanism and chemical induced mechanism.

Pathophysiologic hepatic damage

Disruption of the hepatocyte

Covalent binding of the chemical agent and toxicant to intracellular proteins can cause a decrease in ATP levels, leading to actin disruption. Disassembly of actin fibrils at the surface of the hepatocyte causes blebs and rupture of the membrane. [10]

Disruption of the transport proteins

Chemical agents and toxicants that affect transport proteins at the canalicular membrane can interrupt bile flow. [11] Loss of villous processes and interruption of transport pumps such as multidrug resistance-associated protein-3 prevent excretion of bilirubin, causing cholestasis.

Cytolytic T-cell activation

Covalent binding of chemical agents and toxicants to the P_{450} enzyme acts as an immunogen, activating T cells, cytokines and stimulating a multifaceted immune response. [12]

Apoptosis of hepatocytes

Activation of the apoptotic pathway by the tumor necrosis factor-alpha receptors (TNF-α) of Fas may trigger the cascade of intercellular caspases, which results in cell death. [13]

Mitochondrial disruption

Certain chemical agents inhibit mitochondrial function by a dual effect on both beta-oxidation energy productions by inhibiting the synthesis of nicotinamide adenine dinucleotide and flavin adenine dinucleotide, resulting in decreased ATP production. [14]

Bile duct injury

Toxic metabolites excreted in bile may cause injury to the bile duct epithelium.

Chemical induced hepatic damage

Liver disorders are mainly caused by toxic chemicals, such as antibiotics chemotherapeutic agents, peroxidised oil, aflatoxin, CCl_4, chlorinated hydrocarbons etc. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and by generation of reactive oxidative intermediates in liver. [15]

Direct or intrinsic or predictable drug reactions

Drug or one of its metabolites that fall into this category is either cause reproducible direct toxicity to the liver or lowers the host defense mechanism. The adverse effects occur in most individuals who consume them in dose-dependent manner *e.g.* carbon tetrachloride.

Indirect or unpredictable idiosyncratic drug reactions

Drugs that fall into this group cause immune mediated toxicity, which is independent of concentrations. The drug or one of its metabolites acts as a hapten and induces hypersensitivity in the host. The hepatotoxicity by this group of agents does not occur regularly in all individuals and the effects are usually not dose related *e.g.* acetaminophen. The drugs causing particular type of liver diseases are tabulated in Table 1.

Table 1: Drug induced liver diseases

LIVER DISEASE	AGENTS
Acute viral hepatitis	Acebutolol, Indomethacin, Phenylbutazone, Allopurinol, Isoniazid, Phenytoin, Atenolol, Ketoconazole, Piroxicam, Carbamazepine, Quinine, Diltiazem, Naproxen, Ranitidine, Enflurane, Para-aminosalicylic acid, Sulfonamides, Ethambutol, Penicillins, Sulindac, Labetalol, Probenecid, Cimetidine, Maprotiline, Pyrazinamide, Dantrolene, Metoprolol, Quinidine, Diclofenac, Mianserin, Ethionamide, Phenelzine, Tricyclic antidepressants, Halothane, Phenindione, Valproic acid, Ibuprofen, Phenobarbital, Verapamil
Acute fatty liver infiltration	Adrenocortical steroids, Phenothiazines, Sulfonamides, Antithyroid drugs, Phenytoin, Tetracyclines, Isoniazid, Salicylates, Valproic acid, Methotrexate
Cholestatic jaundice	Actinomycin D, Chlorpropamide, Erythromycin, Amoxicillin/Clavulanate, Cloxacillin, Flecainide, Azathioprine, Cyclophosphamide, Flurazepam, Captopril, Cyclosporine, Flutamide, Carbamazepine, Danazol, Glyburide, Carbimazole, Diazepam, Gold, Cephalosporins, Disopyramide, Griseofulvin, Chlordiazepoxide, Enalapril, Haloperidol, Ketoconazole, Norethandrolone, Sulfonamides, Mercaptopurine, Oral contraceptives, Tamoxifen, Methyltestosterone, Oxacillin, Thiabendazole, Nifedipine, Penicillamine, Tolbutamide, Nitrofurantoin, Phenothiazines, Tricyclic antidepressants, Nonsteroidal, Phenytoin, Troleandomycin, Anti-inflammatory drugs, Propoxyphene, Verapamil
Liver granulomas	Gold, Phenytoin, Aspirin, Hydralazine, Procainamide, Carbamazepine, Isoniazid, Guinidine, Chlorpromazine, Quinidine, Nitrofurantoin, Sulfonamides, Diltiazem, Penicillin, Tolbutamide, Disopyramide, Phenylbutazone
Chronic active hepatitis	Acetaminophen, Dantrolene, Methyldopa, Isoniazid, Nitrofurantoin
Liver cirrhosis or fibrosis	Methotrexate, Terbinafine HCI, Nicotinic acid
Chronic cholestasis	Chlorpromazine/valproic acid (combination), Imipramine, Thiabendazole, Phenothiazines, Tolbutamide, Chlorpropamide/Erythromycin (combination), Phenytoin
Liver tumors	Anabolic steroids, Oral contraceptives, Thorotrast, Danazol, Testosterone

MODE OF ACTION OF LIVER TOXICANTS USED IN EXPERIMENTAL PHARMACOLOGY

Paracetamol

Paracetamol is chemically characterized as N-acetyl-para-aminophenol, which is also known as acetaminophen. Acetaminophen is a safe and effective analgesic in recommended dose. However, acetaminophen is also well

characterized, dose dependent hepatotoxin that can lead to life-threatening acute liver failure when excessive doses are ingested. [16] The hepatotoxic mechanism include the formation of a reactive metabolite, presumably N-acetyl-p-benzoquinamine (NAPQI) through cytochrome P_{450} pathway [17], which is quickly conjugated with hepatic glutathione to yield a harmless product called mercapturic acid. However, after acetaminophen overdose, the capacity for glucuronidation and sulfation is exceed with the formation of excess NAPQI via cytochrome P450 2E1. This intern lead to depletion in glutathione, excess of NAPQI binds to hepatic cell protein and DNA resulting in mitochondrial dysfunction [18] and development of acute hepatic necrosis.

Several P_{450} enzymes such as P450 2E1 play an important role in acetaminophen bioactivation to NAPQI. [19] Studies demonstrated that acetaminophen induced hepatotoxicity can be modulated by substances that influence cytochrome P_{450} activity. [20] Paracetamol induced hepatotoxicity causes rise in SGOT, SGPT, ALP and bilirubin with extensive vascular degenerative changes and centrilobular necrosis in hepatocytes (Fig. 1).

Ethanol

Ethanol is an alcohol that is used commonly as a solvent in medications. Toxicity occurs when an excessive amount is ingested. Alcohol mostly metabolized in the liver through a series of chemical reactions known as oxidation reactions. In the predominant biological pathway for alcohol metabolism, known as alcohol dehydrogenase pathway, the enzyme alcohol dehydrogenase converts alcohol to a toxic intermediate substance, acetaldehyde [21] by removing two atoms of hydrogen from each alcohol molecule. Then a second enzyme, aldehyde dehydrogenase, quickly converts acetaldehyde to acetate [22] by again removing hydrogen and adding oxygen. A secondary pathway of alcohol metabolism is microsomal ethanol-oxidizing system (MEOS). MEOS is activated by long-term heavy alcohol consumption. [23] The MEOS pathway involves the enzyme cytochrome P450 2E1 or CYP 2E1 that strips hydrogen away from alcohol to produce acetaldehyde. [24] In both of these pathways, more markedly in the MEOS pathway-oxidation reactions spawn highly unstable free oxygen radicals.

Normally body deploys molecules called antioxidant to clear oxygen radicals from the liver. However heavy alcohol use not only heightens the production of oxygen radicals but also depletes the supply of antioxidants in the body, creating an imbalance between oxygen radicals and antioxidants. This imbalance is known as oxidative stress which damages liver cell membranes and mitochondria. When oxidative stress is chronic, it contributes to necrosis and liver fibrosis. In addition to its direct effects on the liver, oxidative stress appears to stimulate autoimmune reactions that further damage liver cells. [25] In ethanol induced hepatotoxicity there is significant rise in SGOT, SGPT, ALP and total bilirubin and reduction in total protein, albumin and total cholesterol. The rats treated with ethanol show hepatic cords, fatty infiltration, mesenchymal hyperplasia, fibrosis (Fig. 2a) and fatty infiltration of hepatocytes, hyperplasia of connective tissue, and early manifestation of cirrhosis (Fig. 2b).

Thioacetamide

Thioacetamide is thiono-sulfur containing compound having the fungicidal property. Reif et al 1999 reported that intraperitoneal injection of thioacetamide produce potent hepatotoxicity and carcinogenesis. [26] Chronic administration of thioacetamide produces liver cirrhosis. Thioacetamide is metabolized by cytochrome P_{450} enzymes of liver microsomes and is converted to a toxic intermediate called thioacetamide S- oxide due to oxidation process. [27] Thioacetamide S-oxide induced oxidative stress in the hepatic cells. [28] It is responsible for the changes in cell permeability, increase intracellular concentration of Ca^{++}, increase in nuclear volume, enlargement of nucleoli and also inhibits mitochondrial activity which leads to cell death and severely affecting those cells which are located in the perivenous acinus region. [29] Thioacetamide hepatotoxicity showed significant rise in SGOT, SGPT, ALP and total bilirubin and decrease in total protein. Thioacetamide causes perilobular hepatocyte necrosis, inflammation, infiltration of leukocytes with cytoplasmic vaculation (Fig. 3).

Isoniazid

Isoniazid is chemically isonicotinyl hydrazine that is used to prevent and treat tuberculosis. Isoniazid can cause adverse effects on the liver, ranging from mild transient elevations in aminotransferases to overt hepatitis. N-acetyltransferase (NAT2) metabolizes isoniazid to acetyl isoniazid, which is then hydrolyzed to acetyl hydrazine. [30] Acetyl hydrazine is further metabolized by CYP 2E1 to produce hepatotoxic derivatives. Metabolic oxidation of acetyl hydrazine leads to formation of a reactive acylating species that bind covalently to microsomal protein. Acetyl hydrazine and hydrazine act as acylating agents by binding with the liver cell micromolecules, causing hepatocyte injury. [31] Isoniazid increases SGOT, SGPT, ALP and bilirubin, while decreasing total protein and albumin. The rats treated with isoniazid shows hepatocellular disintegration and vacuolation in centrilobular region (Fig. 4).

Carbon tetrachloride (CCl_4)

Liver toxicant CCl_4 causes lipid peroxidative degradation of biomembrane, which is one of the principle causes of hepatotoxicity. [32] In liver CCl_4 is biotransformed by cytochrome P_{450} to produce its active metabolite trichloromethyl free radical (CCl_3*) [33], which binds to the macromolecule and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxide which intern gives toxic aldehyde that causes damage to liver. Secondary mechanisms link carbon tetrachloride metabolism that could promote the generation of toxic products arising directly from carbon tetrachloride metabolism or from peroxidative degeneration of membrane lipids. The possible involvement of toxic intermediates radical species are such as trichloromethyl (CCl_3*), trichloromethylperoxy ($OOCCl_3$*) and chlorine (Cl*) free radicals as well as phosgene and aldehydic products of lipid peroxidation.

This radical can bind to cellular molecules (nucleic acid, protein and lipid) impairing crucial cellular processes such as lipid metabolism, with the potential outcome of fatty degeneration (steatosis). This radical can also react with oxygen to form a highly reactive specie, trichloromethylperoxy radical (CCl_3OO*). CCl_3OO* initiates the chain reaction of lipid peroxidation which attacks and destroys polyunsaturated fatty acids, in particular those associated with phospholipids. This affects the permeability of mitochondrial, endoplasmic reticulum and plasma membranes resulting in the loss of cellular calcium

sequestration and homeostasis, which can contribute heavily to subsequent cell damage. Carbon tetrachloride significantly increases SGOT, SGPT, ALP and total bilirubin where as decreasing total protein, albumin and total cholesterol. Rat liver tissue treated with carbon tetrachloride shows hepatocellular necrosis, fatty vacuole and microvesicular fatty changes (Fig. 5).

Galactosamine

Galactosamine is a hexosamine derived from galactose. It causes liver injury via the generation of free radicals and depletion of UTP nucleotides. Galactosamine produces the hepatotoxic effect by selectively reducing the uridine pool in hepatocytes. This intern inhibits mRNA and protein synthesis, alters the composition of cellular membranes and finally leads to cellular damage as a result of lipid peroxidation. [34] The hepatocyte death is represented as apoptosis and subsequently necrosis. [35] Other mechanism of galactosamine hepatotoxicity stated that galactosamine increases intestinal permeability and subsequently facilitates bacterial translocation to the liver. [36] Lipopolysaccharides activate kupffer cells to secrete tumor necrosis factor-α [37], which raises expression of intercellular adhesion molecule 1 in endothelial cells [38] and this promotes the adhesion of polymorphonuclear cells to vascular [39] and hepatic endothelial cells [40], leading to polymorphonuclear infiltration and hepatocyte damage. Galactosamine induces rise in SGOT, SGPT and total bilirubin where as decrease in total protein. Galactosamine shows pathological changes like moderate degeneration and necrosis of hepatocyte (Fig. 6).

Cadmium

Cadmium metals and metalloids affect almost every organ of the body, including the liver. One such metal is cadmium, which is of concern because of its increasing prevalence as an environmental contaminant. [41] Prolonged exposure to cadmium results in injury to the liver. A large bolus dose of cadmium causes injury to a number of tissues, including the liver. [42] Cadmium induces oxidative damage in different tissues by enhancing peroxidation of membrane lipids in tissues and altering the antioxidant systems of the cells. The peroxidative damage to the cell membrane may cause injury to cellular components due to the interaction of metal ions with the cell organelles. [43] Cadmium depletes glutathione and protein bound sulfhydryl groups resulting in enhanced production of reactive oxygen species such as superoxide ions, hydroxyl radicals, and hydrogen peroxides. These reactive oxygen species result in increased lipid peroxidation, [44] hepatic congestion, ischemia and hypoxia. The resultant ischemic hypoxia leads to neutrophil infiltration, kupffer cell activation, and inflammation which could potentially contribute to the widespread hepatocellular apoptosis and necrosis. [45] Cadmium causes increase in serum concentrations of urea, creatinine, glucose, AST, acid phosphatase, alkaline phosphatase, alanine transaminase, aspartate transaminase, and serum bilirubin where as reducing serum protein and tissue protein concentration. Cadmium treated rat shows the histopathological changes like periportal inflammation (Fig. 7a), microvesicular steatosis and balloon degeneration (Fig. 7b).

Aflatoxin B1 (AFB1)

AFB1 is a naturally occurring fungal toxin that causes both acute hepatotoxicity and liver carcinoma in humans and animals. AFB1 produces the hepatotoxicity through the formation of adducts with DNA, observed both in vitro and in rat liver. [46] These adducts are derived from highly reactive exo-epoxide metabolites of AFB1, as a result of oxidation reactions within the liver. [47] Several cytochromes P_{450} have been implicated in this activation and in human these were identified as CYP 1A2 and CYP 3A4. [48] The formation of adducts between the AFB1-exo-epoxide and the N7 of guanine bases in DNA leads to mutations and is strongly associated with the presence of preneoplastic lesions, characterized as GST-P-positive foci, providing a direct link between DNA damage, mutagenesis and carcinogenesis. AFB1 causes acute toxicity as well as carcinogenicity in rats and as observed in early studies. [49] Acute toxicity was initially attributed to mainly genotoxic effects of the epoxide; dependent on the formation of DNA adducts which at high levels lead to cell death. However, a dialdehyde metabolite of AFB1 that rapidly forms from the epoxide, can form adducts with proteins and these were proposed to contribute to the acute toxicity. [50] In addition, such cellular necrotic damage caused by AFB1 dialdehyde may lead to compensatory liver hyperplasia and by so doing may promote the incorporation of mutations into the DNA of dividing cells and contribute towards carcinogenicity initiated by the AFB1-exo-epoxide. [51] AFB1 increases serum concentrations of SGOT, SGPT, alkaline phosphatase and bilirubin, and decrease in serum cholesterol. The prominent gross pathologic and histopathologic changes in the liver are hemorrhage, necrosis, and massive accumulation of lipid (Fig. 8).

Allyl alcohol

The toxicity of allyl alcohol is considered to be mediated via acrolein, which is generated from allyl alcohol by the enzyme alcohol dehydrogenase. [52] Acrolein is a highly toxic member of a class of α-β unsaturated aldehydes, namely 2-alkenals. [53] Acrolein, is a powerful electrophile and reacts with nucleophiles such as sulphydryl groups. [54] The reaction is accelerated by the activity of cytosolic glutathione S-transferase [55] to form an aldehyde-GSH adducts which is metabolized to acrylic acid. Glutathione is primarily involved in the reaction, which result in a depletion of cellular glutathione stores, followed by hepatocellular necrosis. [56] Allyl alcohol induces increase in SGOT, SGPT and total bilirubin where as decrease in total protein. The rats treated with allyl alcohol shows necrosis around branches of the central hepatic vein and presence of a large amount of nuclear debris (Fig. 9).

Halothane

Halothane is chemically 2-bromo-2-chloro-1-1-trifluoroethane. It has been used widely as an inhaled anaesthetic [57] and as liver toxicant in animal models. It is well established that halothane is metabolized in the liver as a lipophilic xenobiotic to hepatotoxic intermediates by monooxygenases through the cytochrome P450 2E1 system. [58] Thus, halothane anaesthesia causes hepatocellular necrosis, destruction of the lipid-protein interactions in human erythrocyte membranes, decrease in activities of membrane enzymes and alteration of cerebral glucose-6-phosphate dehydrogenase (E.C.1.1.1.49, G6PDH) activities. [59] Halothane treated rat liver shows extensive centrilobular necrosis and denaturation (Fig. 10).

BIOCHEMICAL ALTERATIONS IN HEPATIC DAMAGE

The biochemical changes in the blood reflect the histologic pattern of toxicity in hepatic injury. Biochemical alterations provide a static assessment of the degree of liver injury.

Serum aminotransferase enzymes

Serum concentrations of aspartate aminotransferase (AST) or glutamate oxaloacetate transaminase (SGOT) and alanine aminotransferase (ALT) or glutamate pyruvate transaminase (SGPT) are the most commonly used biochemical markers of hepatocellular necrosis. [60] These serum activities presumably increase as a result of cellular membrane damage and leakage. [61] Serum levels of SGOT and SGPT are increased on damage to the tissues producing them. Serum aminotransferase activities are increased in all types of hepatic injury. Thus serum estimation of SGPT which is fairly specific for liver tissue is of greater value in liver cell injury, whereas SGOT level may raise in acute necrosis or ischemia of other organs such as the myocardium, besides liver cell injury. The highest increases are observed with acute hepatocellular injuries, such as xenobiotic-induced necrosis or acute viral hepatitis. [62] Serum activities are generally within the reference interval or only slightly increased in alcoholic liver disease. [63]

Serum alkaline phosphatase

Serum alkaline phosphatase is produced by many tissues especially bone, liver, intestine, placenta and is excreted in the bile. Serum alkaline phosphatase increases to some extent in most types of liver injury. Bile acids induce alkaline phosphatase synthesis and exert a detergent effect on the canalicular membrane, allowing leakage into serum. [61, 64] The highest concentrations are observed with cholestatic injuries. [60]

Serum total protein and albumin

Proteins form the major portion of dissolved substances in the plasma. Liver cells synthesize albumin, fibrinogen, prothrombin, alpha-1-antitrypsin, haptoglobin, ceruloplasmin, transferrin, alpha fetoproteins and acute phase reactant proteins. The blood levels of these plasma proteins are decreased in extensive liver damage.

Serum albumin, the major plasma protein synthesized in the human liver, is a clinically useful marker of hepatic synthetic function. [60] Alcoholic cirrhosis with or without accompanying ascites generally lowers serum albumin concentrations. [61]

Serum total and direct bilirubin

The serum bilirubin level is one of the best tests of liver function. Bilirubin is the metabolic product of the break down of heme derived from senescent red blood corpuscles. An increase in urinary bilirubin is nearly always indicative of a corresponding increase in the serum, attributable to intrahepatic or extrahepatic cholestasis. The degree of increase in serum bilirubin values has prognostic significance in chronic liver injuries, but not in acute injuries. [65] If the direct or conjugated bilirubin is low, while the total bilirubin is high, this reflects liver cell damage or bile duct damage.

Serum bile acids

Measurement of bile acid concentrations is a good indicator of hepatobiliary function. An increase in serum bile acid concentrations in fasting is highly specific for liver injury and serves to exclude congenital or hemolytic causes of hyperbilirubinemia. [66] The greatest increases are observed in acute viral hepatitis or extrahepatic cholestasis. [60]

Serum lipid profile

Cholesterol is the main lipid found in the blood, bile and brain tissues. Liver metabolizes the cholesterol and it is transported to the blood stream by lipoproteins. Decreased levels are found in malabsorption, malnutrition, hyperthyroidism, anemia and liver diseases.

Triglycerides are simple lipids formed in the liver by glycerol and fatty acids. They are transported by very low density lipoprotein (VLDL) and low density lipoprotein (LDL). They constitute about 90 % of fat, stored as source of energy in the tissue and plasma.

Serum γ- glutamyl transferase

The measurement of serum γ-glutamyl transferase is a frequently used parameter of liver diseases. The serum enzyme originates from liver and is cleared from the circulation by the galactose receptor in liver. The rate of uptake will thus vary with the amount of terminal galactose residues on the enzymes carbohydrate moiety. The enzyme is inducible by chronic alcohol use liver abscess and by drugs such as phenytoin. [67]

Serum 5'-nucleotidase

5'-Nucleotidase (5NT) is an intrinsic membrane glycoprotein produced by the liver present as an ectoenzyme in a wide variety of mammalian cells, hydrolyzes 5'-nucleotides to their corresponding nucleosides. Despite its ubiquitous distribution, serum concentrations of 5NT appear to reflect hepatobiliary disease with considerable specificity. [68] The primary utility of serum 5'-nucleotidase activities is in the diagnosis of cholestatic liver injury in childhood or pregnancy.

Serum lactic dehydrogenase

Lactate dehydrogenase, also called lactic dehydrogenase (LDH), is an enzyme found in the cells of many body tissues, including the heart, liver, kidneys, skeletal muscle, brain, red blood cells, and lungs. It is responsible for converting muscle lactic acid into pyruvic acid, an essential step in producing cellular energy. Lactic dehydrogenase is present in almost all body tissues, so the LDH test is used to detect tissue alterations and as an aid in the diagnosis of heart attack, anemia, and liver disease.

Alpha-fetoprotein

Alpha-fetoprotein (AFP) is a protein normally produced by the fetal liver and is present in the fluid surrounding the fetus (amniotic fluid), and crosses the placenta into the mother's blood. At birth, infants have relatively high levels of AFP, which fall to normal adult levels by the first year of life. AFP probably has no normal function in adults. The most widely used biochemical blood test for liver cancer is AFP.

Mitochondrial antibodies

Anti-mitochondrial antibodies (AMA) represent a heterogeneous mixture of antibodies to at least 9 different antigens, which are designated M1-M9. Primary biliary sclerosis (PBC) is an autoimmune disease that causes destruction of intrahepatic bile ducts. PBC is often associated with other autoimmune disorders, particularly Sjogren syndrome. Anti-mitochondrial antibodies (AMA) are found in almost all patients with PBC and are considered the serological hallmark of the disease. AMA is useful diagnostically in distinguishing primary biliary cirrhosis from other types of chronic liver disease. [69]

HISTOPATHOLOGICAL ALTERATION IN HEPATIC DAMAGE

The liver reacts with eight different types of responses to injury towards variety of metabolic, toxic, microbial,

circulation and neoplastic insults. [70] Damage from toxic or immunologic insult may cause hepatocytes to take on a swollen and edematous appearance (ballooning degeneration) with irregularly clumped cytoplasm and large clear spaces. Alternatively, retained biliary material may impart a diffuse foamy swollen appearance to the hepatocyte (foamy degeneration). Substances may accumulate in viable hepatocytes including iron and copper. Accumulation of fat droplets within hepatocytes is known as 'steatosis' and appear in such conditions as alcoholic liver disease and acute fatty liver of pregnancy. A single large droplet that displaces the nucleus (macrovesicular steatosis) may be seen in the alcoholic liver or in the liver of obese or diabetic individuals.

Necrosis is defined as focal death along with degradation of tissue by hydrolytic enzymes liberated by cells. Various agents such as hypoxia, chemical, physical agents, microbial agents and immunological injury can cause necrosis. Two essential changes bring about irreversible cell injury in necrosis is cell digestion by lytic enzymes and denaturation of proteins. These processes are morphologically identified by characteristic cytoplasmic and nuclear changes in necrotic cell. The cytoplasm appears homogeneous and intensely eosinophilic. Occasionally, it may show vacuolation or dystrophic calcification. The nuclear changes include condensation of nuclear chromatin which may either undergo dissolution or fragmentation into many granular clumps.

Centrilobular necrosis frequently exhibits a zonal distribution. The most obvious necrosis of hepatocytes immediately around the terminal hepatic vein, an injury that is characteristic of ischemic injury occurred for a number of drug and toxic reactions. A variable mixture of hepatocellular death and inflammation is encountered. The hepatocyte necrosis may be limited to scattered cells within hepatic lobules. Bridging necrosis is more severe inflammatory injury; necrosis of continuous hepatocytes may span adjacent lobules in a portal to portal, portal to central and central to central fashion. Submassive necrosis of entire lobules or most of the liver is usually accompanied by hepatic failure. With disseminated candidal or bacterial infection, macroscopic abscesses may occur. In fat necrosis, the necrosed fat cells have cloudy appearance, surrounded by an inflammatory reaction. Formation of calcium soaps is identified in the tissue sections as amorphous, granular and basophilic material. Microscopically, fibrinoid necrosis is identified by brightly eosinophilic, hyaline-like deposition in the vessel wall or on the hepatocytes. All the histopathological changes characteristic of different types of liver diseases is tabulated in Table 2.

COMMON LIVER FUNCTION TESTS

Liver function tests routinely combine markers of function (albumin and bilirubin) with markers of liver damage (alanine transaminase, alkaline phosphatase, and γ-glutamyl transferase). Abnormalities in liver enzyme activities give useful information about the nature of the liver insult: a predominant rise in alanine transaminase activity (normally contained within the hepatocytes) suggests a hepatic process. These tests can also distinguish between acute and chronic liver disorders and between hepatitis and cholestasis. The most commonly performed blood tests include:

Serum glutamate oxaloacetate transaminase (SGOT) test

This enzyme is released from damaged liver, heart, muscle, kidney or brain cells. Its normal serum level is up to 46 IU/L at 37°C. [15] SGOT levels are 10 to 200 fold elevated in patients with acute hepatic necrosis, viral hepatitis, CCl_4 and drug induced poisoning. SGOT levels are also elevated by 10 fold in patients of post hepatic jaundice, intra hepatic cholestasis and less than 10 fold in alcoholic and hepatic steatosis. [71]

Serum glutamate pyruvate transaminase (SGPT) test

This enzyme is released from damaged liver cells. Normal serum level of SGPT is up to 49 IU/L at 37°C and its levels are very high in patients of viral hepatitis and hepatic necrosis, 10 to 200 fold higher in patients of post hepatic jaundice, intrahepatic cholestasis and below 10 fold in patients of metastatic carcinoma, cirrhosis and alcoholic hepatitis. [72]

Serum alkaline phosphates test

Elevated levels of alkaline phosphatase, an enzyme found in the bile, usually indicate an obstruction of bile flow, liver injury, or certain cancers (Dial, 1995). Elevation in normal serum alkaline phosphatase (range 3-13 King Armstrong units/dl or 25-85 IU/dl) activity is found in diseases of bone, liver and in pregnancy. In the absence of bone disease or pregnancy, an elevated serum alkaline phosphatase level generally reflects hepatobiliary disease. The greatest elevation (3-10 times of normal) occurs in biliary tract obstruction. Slight to moderate increase is seen in parenchymal liver diseases such as hepatitis, cirrhosis and metastatic liver disease. [15]

Serum total protein and albumin test

Routinely estimated total proteins are in the normal range of 5.5 to 8 g/dl. The blood levels of plasma protein are decreased in extensive liver damage. Albumin (normal range 3.5 to 5.0 g/dl) synthesized in the liver constitutes a major part of the total proteins in the body and the other part being globulin. A low serum albumin concentration suggests chronic liver disease. [73] Hypoalbuminaemia may occur in liver diseases caused by significant destruction of hepatocytes. Hyperglobulinaemia may be present in chronic inflammatory disorders such as in cirrhosis and in chronic hepatitis.

Serum total and direct bilirubin test

Each day about 7.5 g of hemoglobin is catabolized with the corresponding production of 250 mg of bilirubin. Normally 0.25 mg/dl of conjugated bilirubin is present in the blood of an adult. Normal range for total bilirubin is from 0.2 to 1.2 mg/dl. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, hemolysis and in defects of hepatic uptake and conjugation of bilirubin such as in Gilberts disease. [15] Elevated levels of bilirubin often indicate an obstruction of bile flow or a defect in the processing of bile by the liver. If the direct or conjugated bilirubin is low, while the total bilirubin is high, this reflects liver cell damage or bile duct damage. [74]

Serum bile acids

Fasting bile acids concentrations in excess of 15 µmol/L can be the result of hepatobiliary disease. The probability of hepatobiliary disease is high if fasting bile acid concentration is greater than 25-30 µmol/L.

Serum lipid profile test

Liver toxicants cause disturbances in synthesis and metabolism of triglycerides, cholesterol and lipoproteins, thus damaging the basic resource for living cells. Cholesterol and bile salts are synthesized by liver cells thus liver intoxication decreases level. Normal range of cholesterol levels are up to 200 mg/dl or lower for a total count, but it is

Table 2: Characteristic histopathological changes in different types of liver diseases

LIVER DISEASE	CAUSES	CHARACTERISTIC HISTOPATHOLOGICAL CHANGE
Hepatitis		
Acute	Alcohol abuse and hepatotropic viral infection	Cell injury in centrilobular zone, ballooning degeneration, apoptosis, dropout necrosis and bridge necrosis, inflammation infiltrate in portal tracts and kupffer cell hyperplasia
Chronic active	Autoimmune chronic active hepatitis, hepatitis B and C virus, hepatitis B virus with delta infection, Wilson's disease, haemochromatosis, alcohol and alpha-1-antitrypsin deficiency	Intense portal inflammatory infiltrate spills over hepatic parenchyma, piecemeal necrosis, and bridging necrosis between adjacent triads, progressive fibrosis beginning in the portal triads and radiating into the liver parenchyma
Chronic persistent	Hepatitis B and C virus and combined hepatitis B and delta infection	Lymphocyte and macrophage infiltration is limited to the portal triads and no significant hepatocyte necrosis
Chronic liver disease		
Liver cirrhosis	Alcohol abuse, hepatitis, viruses, certain drugs, chemicals, bile duct obstruction, autoimmune diseases, obstruction of blood outflow from liver, alpha-1-antitrypsin deficiency, high blood tyrosine levels at birth, diabetes, malnutrition and Wilson's Disease or hemochromatosis	Diffuse damage to hepatic parenchyma cells, with nodular regeneration, fibrosis, and disturbance of normal architecture
Liver fibrosis	Infection, inflammation and injury	Pericellular, periveular fibrosis and web appearance
Autoimmune liver disorders		
Hemochromatosis	Absorption of too much iron from food	No histopathological change
Wilson's disease	Retention of too much copper in the liver	No histopathological change
Alcohol-induced liver disease		
Fatty liver	Excessive consumption of alcohol	Swelled hepatocyte, deposition of large fat droplets and formation of microvesicles, fatty changes in centrizone and mid-zone and diffuse change Collagen laid down around the cells and hepatic veins - pericellular and perivenular fibrosis.
Alcoholic hepatitis	Excessive consumption of alcohol	Ballooning degeneration and necrosis of hepatocytes near the central veins, retention of secretory proteins and water, causing cell swelling and formation of Mallory bodies
Alcoholic cirrhosis	Excessive consumption of alcohol	Hepatocytes may show slight increased cytoplasmic haemosiderin
Congenital liver defects		
Biliary atresia	Absense or abnormally developed bile ducts	No histopathological change
Choledochal cyst	Malformation of the hepatic duct	No histopathological change
Benign Liver tumors		
Hemangioma	Birth defect	Tumor is found as hemorrhagic lesion composed of blood spaces of various sizes covered with single layer of endothelial cells separated by thin fibrous stroma
Focal nodular hyperplasia	Congenital vascular malformation or vascular injury	Central stellate scar with radiating fibrous septa, regions of nodular hepatocellular proliferation separated by radiating bands and surrounding myxomatous scar
Hepatocellular adenoma	Use of oral contraceptive pills with a high oestrogen content	Intratumoral hemorrhage, large intratumoral vessels, fatty change and peliosiss
Malignant liver tumors		
Hepatocellular carcinoma	Prolonged infection with Hepatitis B and C virus, alcohol, mycotoxin and chemical carcinogens	Large solid tumor mass separated by vascular space, inconspicuous sinusoids and central cystic space formed by degeneration
Haemangio-sarcoma	Vinyl chloride monomers, thorotrast and arsenic	Cystic degeneration, haemorrhage and necrosis
Hepatoblastoma	Beckwith-Wiedemann syndrome, familial adenomatous polyposis and hemihypertrophy. Inborn errors of metabolism such as tyrosinemia, glycogen storage disease type I, galactosemia and alpha-1-antitrypsin deficiency	Circumscribed tumor having areas of cystic degeneration, haemorrhage and necrosis

important to check HDL and LDL levels for a better analysis. Fatty degeneration of the liver causes increased triglyceride (normal range >150 mg/dl) content in the blood.

γ- glutamyl transferase test

Gamma glutamyl transferase (γ -GT) also known as γ-glutamyl transpeptidase is a microsomal enzyme with wide tissue distribution. This enzyme is produced by the liver, pancreas, kidneys and released into the blood when these organs are injured. The normal γ- GT serum level is upto 26 U/L. In alcoholics with liver abscess, it is increased by 2-5 times.

5-Nucleotidase test

Normal range of 5-Nucleotidase is 2 to 17 U/L. The liver releases this enzyme when injured due to bile duct obstruction or impaired bile flow. Greater than normal values indicate liver cell destruction, liver ischemia, necrosis, hepatitis, cholestasis or liver tumor.

Lactic dehydrogenase test

Reference ranges for total LDH vary from laboratory to laboratory. Normal values are also higher in childhood. For adults, in most laboratories, the range can be up to approximately 200 U/L, but is usually found within 45-90 U/L. When disease or injury affects tissues containing LDH, the cells release it into the bloodstream, identified as higher than normal levels. The LDH is also elevated in heart attack, diseases of the liver, in certain types of anemia, and in cases of excessive destruction of cells, as in fractures, trauma, muscle damage and shock.

Alpha-fetoprotein test

In adults, high blood levels (over 500 ng/ml) of AFP are seen in only three situations like hepatocellular carcinoma, germ cell tumors and metastatic cancer in the liver. Also, pregnant women carrying babies with neural tube defects may have high levels of AFP.

Mitochondrial antibodies test

AMA are present in less than 1 % of normal people and in less than 5% of patients with systemic lupus erythematosus,

rheumatoid arthritis and other autoimmune diseases. Patients with extrahepatic biliary obstruction, Wilson's disease, hemochromatosis, and alcoholic cirrhosis rarely have elevated titers. The presence of mitochondrial antibodies remains a useful diagnostic tool in the differential diagnosis between primary biliary cirrhosis and extrahepatic biliary obstruction.

Prothrombin time (PTT) test

This test measures the time it takes for blood to clot. Blood clotting requires vitamin K and coagulation factors like II, V, VII, and IX synthesized in the liver. Liver cell damage and bile flow obstruction can both interfere with proper blood clotting.

EXPERIMENTAL PHARMACOLOGICAL STUDIES IN ANIMAL LIVER

To investigate and evaluate hepatoprotective substance, it is customary to subject animals to a range of toxic agents. These hepatotoxicants include carbon tetrachloride, galactosamine, thioacetamide, ethanol, aflatoxin B1, alpha amanitine, phalloidin, cadmium, paracetamol, hydrazine, halothane, isoniazid etc that causes damage of rat liver, resulting in biochemical and histopathological changes. Different toxicants used for experimental liver damage with dose range, route, vehicle and detailed schedule of treatment are presented in Table 3.

Table 3: Different toxicants used for experimental liver damage with dose, route, vehicle and detailed schedule of treatment

TOXICANT	VEHICLE	ANIMAL	ROUTE	DOSE	SCHEDULE	REF
Paracetamol	0.5 % CMC or	Wistar albino rat	I.P.	600 mg/kg	Daily treatment for 14 days	75
	0.2% tragacanth	Wistar albino rat	Oral	500 mg/kg to 3 gm/kg	Single dose on 3rd, 7th or 8th day	76, 77
Ethanol	Sterile water	Wistar albino rat	Oral	1.5 gm/kg to 12 gm/kg or 15% v/v or 5 ml/100 gm	30 days to 45 days	78, 79
Thioacetamide	Sterile water or saline	Wistar albino rat	S.C.	100 mg/kg	Single dose on 21st day of drug treatment	80
		Sprauge-Dawley rat	I.P.	50 mg/kg to 350 mg/kg	Single dose on every 24th hr for 3 consecutive days	81, 82
Isoniazid	Sterile water or saline	Rabbit	I.P.	50 mg/kg	Single dose for 11 days	83, 84
Carbon tetrachloride	Olive oil	Wistar albino rat	S.C.	1 ml/kg	Single dose on 7th day or 4 consecutive days	85, 86
		Wistar albino rat	S.C.	0.15 ml/kg	There times a week for 10 weeks	87
Galactosamine	Saline	Swiss albino rat	I.P.	650 mg/kg to 800 mg/kg	Single dose	88, 89
Cadmium chloride	Sterile water or saline	Swiss albino rat	S.C.	3 mg/kg	Every alternate days for 2 weeks	90
		Wistar albino rat	Oral	6 mg/kg	One month to three months	91
		Sprague-Dawley rat	I.P.	3.5 mg/kg	Single dose on 7th day of treatment	92
		Sprague-Dawley rat	I.V.	4 mg/kg	Single dose on 3rd day of treatment	93
Aflatoxin B1	8% dimethyl-sulfoxide	Sprague-Dawley rat	I.P.	1 mg/kg to 2 mg/kg	Single dose	94, 95
Allyl alcohol	Sterile water or saline	Wistar albino rat	Oral	0.4 mg/kg	Single dose of toxicant administered on 3rd day	96
		Swiss albino mice	I.P.	75 µl/kg	Single dose	97
Halothane	21% with O_2	Guinea pig	Inhalation	1% v/v	Inhalation for 4 hrs	98, 99

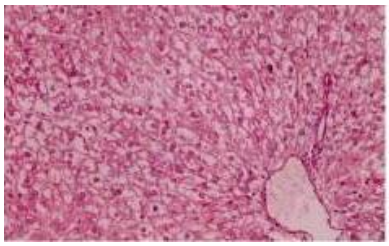

Fig. 1

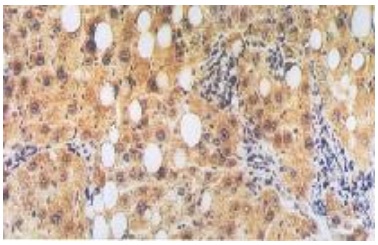

Fig. 2a

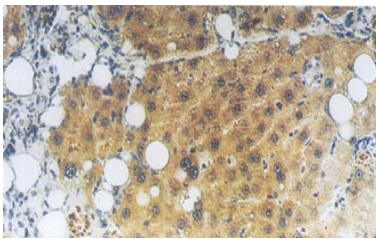

Fig. 2b

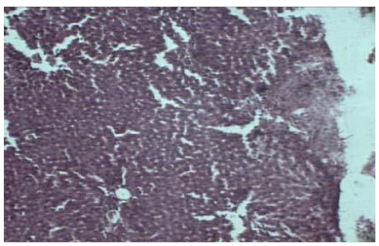

Fig. 3

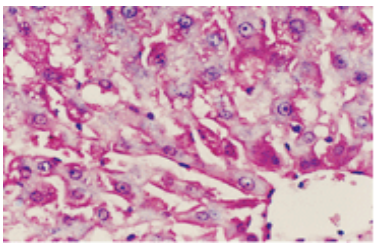

Fig. 4

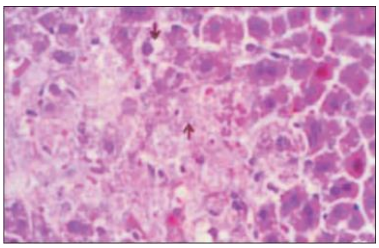

Fig. 5

Fig. 1: Extensive vascular degenerative changes and centrilobular necrosis induced by paracetamol. Fig. 2: Ethanol intoxication induces fatty infiltration, mesenchymal hyperplasia and fibrosis (Fig. 2a), and hyperplasia of connective tissue along with early manifestation of cirrhosis (Fig. 2b). Fig. 3: Thioacetamide causes perilobular hepatocyte necrosis, infiltration of leukocytes with cytoplasmic vaculation. Fig. 4: Isoniazid shows hepatocellular disintegration and vaculation in the centrilobular region. Fig. 5: Carbon tetrachloride treatment shows hepatocellular necrosis, fatty vacuole and microvesicular fatty changes.

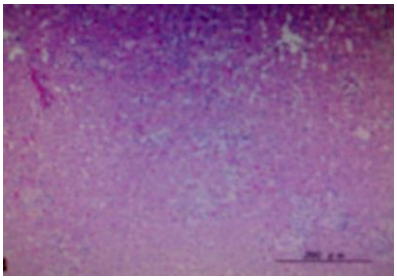

Fig. 6

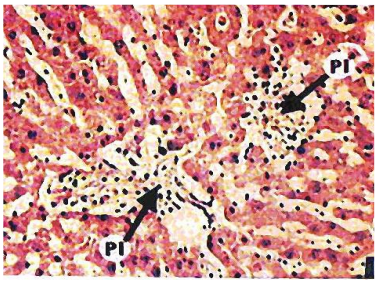

Fig. 7a

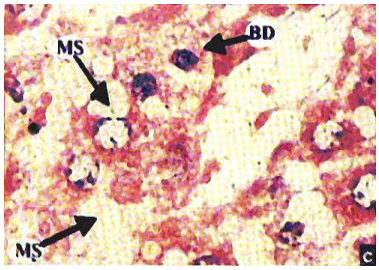

Fig. 7b

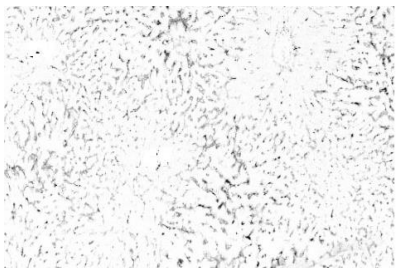

Fig. 8

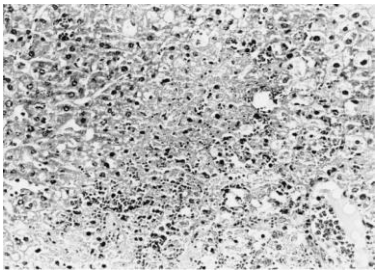

Fig.9

Fig. 10

Fig. 6: Galactosamine shows pathological changes like moderate degeneration and necrosis of hepatocyte. Fig. 7: Cadmium treated rat shows the histopathological changes like periportal inflammation (Fig. 7a), microvesicular steatosis and balloon degeneration (Fig. 7b). Fig. 8: The prominent gross pathologic and histopathologic changes like hemorrhage, necrosis, and massive accumulation of lipid induced by Aflatoxin B1. Fig. 9: The rats liver treated with Allyl alcohol shows necrosis around branches of the central hepatic vein and presence of a large amount of nuclear debris. Fig. 10: Halothane causes extensive centrilobular necrosis and denaturation.

Animals

Hepatotoxicity studies can be carried out in different strains of mice and rats of either sex maintained at uniform laboratory conditions. Animals should be housed at a temperature of 25±2ºC, relative humidity of 50±15 % and 12:12, light: day. All animals are allowed to free access to water and fed with standard commercial pellet rat chaw. The animals are acclimatized for a period of 7 days before performing the experiment. Inhalation anaesthetic induced hepatotoxicity studies are usually done on guinea pigs. Rabbits are choice of species for drug induced chronic hepatotoxicity study.

Methodology

Before the commencement of experiment the animals are kept fasted overnight and then divide into several groups. One group will serve as a vehicle control group, receiving vehicle only. Hepatotoxicant (dissolve in the suitable solvent as depicted in Table 3) is administered to animals of the other groups through the suitable route as per the study protocol. The standard drug is administered to different group serves as positive control and only toxicant is administered to another group designating negative control. The test drug is administered in varying concentration or dose for a specified duration depending on the design of the experiment. At the end of experiment, all the animals are sacrificed after a predecided period (18-48 h after last dose) under light ether anesthesia or by direct cervical dislocation. Biochemical estimation is performed on serum by drawing blood either from carotid artery or through cardiac puncture in hyphenised tube. The blood samples are left to clot at room temperature for atleast 1 h. Serum is separated by centrifugation at 3000 rpm for 20 min at 4°C to carry out the different assay. N some cases the blood can also be collected from retino-bulbar venous plexus under light ether anesthesia for the biochemical estimation. For histopathology liver is removed, washed with infusion of cold saline and weighed. Liver sections are taken from each lobe of liver, which are immediately frozen and stored at -70°C or fixed in 10 % normal formaline until analyzed.

Biochemical estimation

The activities of serum glutamate oxaloacetate transaminase (SGOT or AST) and serum glutamate pyruvate transaminase (SGPT or ALT) are measured in serum according to the methods described by Reitman and Frankel. [100] Lactase dehydrogenase (LDH) activity is measured by the method of Varley. Alkaline phosphatase (ALP) activity is determined according to Kind and King. [101] Total bilirubin and urea levels are estimated according to Malloy and Evlyn. [102] Plasma protein concentration was measured according to the method of Bradford. [103] Hepatic glucose-6-phosphatase is determined in the soluble fraction of liver homogenates according to the described by Harper. [104] Acid phosphatase (ACP) activity in liver was assayed using a Boehringer kit. The activity of albumin and total cholesterol are measured according to the method of Varley. [105] Hepatic lipid peroxidation is assayed by measuring the concentration of malondialdehyde (MDA) in 10% w/v liver homogenate according to the procedure of Okhawa. [106] Glutathione-S-transferase content in liver cytosolic fraction is determined by the method of Ellman. [107]

Histopathological observation

After fixing in 10 % neutral formalin solution, liver tissues are dehydrated with ethanol solution, embedded in paraffin, cut into 5 µm section, stained with haematoxylin-eosin dye and then observed under a photomicroscope. Different hepatotoxicants show the different morphological changes that may be disturbance of normal architecture of parenchyma cells, swelled hepatocyte, and formation of mallory bodies, microvesicles sinusoidal congestion, infiltration of lymphocytes, kupffer cells around the central

vein and loss of cell boundaries. Histopathological changes may also include massive fatty changes, ballooning degeneration, lymphocyte, macrophage infiltration, nodular regeneration, steosis, fibrosis, and tumor formation.

Assessment of liver function

Functional ability of liver is assessed on thiopentone induced sleeping time, bromosulphthalein clearance and viability by trypan blue exclusion test.

Barbiturate induced sleeping time

Hepatic damage can be measured by accessing activity of hepatic microsomal drug metabolizing enzymes. The hepatotoxicant attacks the membranes of smooth and rough endoplasmic reticulum thus reducing the quantity of microsomal enzymes. Intoxicated liver prolongs duration of sleeping time for hexobarbitone, thiopentone, zoxazolamine and pentobarbitone etc in animals. Thiopentone, hexobarbitone, or pentobarbitone induced sleeping time is increased in animals with liver intoxication as the enzyme responsible for metabolism of thiopentone is reduced or destroyed. [108]

Bromosulphthalein uptake test

The liver cells remove the dye, bromosulphthalein (BSP) from the plasma and excrete it into bile. It has been reported that BSP is secreted into bile as mercaptide conjugate of glutathione and cystein, which takes place in liver. Bromosulphthalein clearance test is the most sensitive and dependable method to assess the physiological status of liver function. The test indicates the excretory function of the liver. It is generally agreed that in the passage of BSP from the plasma to the bile, it undergoes storage, metabolism, and excretion by the liver. Concentration of BSP in plasma greater than 5.8 mg/ml is considered as indicative of liver damage after 15 min of 100 mg/kg (i.v.) BSP administration. [109]

BSP clearance rate can be estimated spectrophotometrically *in-vitro* in isolated liver slices following method of Rajan and Subrahmanyam. [110]

Trypan blue exclusion test

Loss of cell viability is most often measured as loss of membrane integrity. This event may be primarily due to necrosis or secondarily due to apoptosis. Trypan blue exclusion is a cell viability assay based on the ability of the liver cells to exclude the trypan blue and uptake of the dye by the dead cells due to alteration in the membrane permeability which can be measured following method of William *et al.*, 1971. [111]

While undertaking hepatoprotective potential assessment of a drug or chemical the study parameters should be well designed covering all aspects of liver functionality. Effectiveness of a particular drug against a specific type of liver disease can only be claimed when it is experimentally proved in preclinical animal studies. Preclinical study reports are also necessary for approval to carry out clinical studies. To study hepatoprotective potential of any herbal product, isolated phytochemical or synthetically developed moiety induction of experimental liver damage is prerequisite. Liver damage can be caused by exposure to a range of toxic chemicals. Toxic liver injury produced by drugs and chemicals may virtually mimic any particular form of naturally occurring liver disease. Dose and duration of exposure with toxicants are also crucial in concern to achievement of acute to chronic type of liver damage. The selection of liver toxicant must depends on targeted nature of liver damage required.

The hepatoprotective potential of any promising drug can be evaluated by treating the experimental animal along with the toxicant and assessing functional parameters of liver. A range of liver function tests is employed for accurate diagnosis of disease prognosis and therapy evaluation. This review precisely compiles the details of different liver toxicants used in experimental pharmacology like, dose, route, and along with mechanism of damage. The literature compiles biochemical alterations and histological characteristic damage of liver cytology in detail. Different functional ability test and viability assessment studies can be referred from the review. This extensively cited and well documented review will definitely help the researcher in liver protective study protocol preparation and cross referencing the published methods.

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